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Problems with the Oxford Multilocus Sequence Typing Scheme for *Acinetobacter baumannii*: Do Sequence Type 92 (ST92) and ST109 Exist?

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Even in the genomic era, multilocus sequence typing (MLST) remains a method of choice for bacterial typing, quickly revealing relationships within and between bacterial lineages. There are two *Acinetobacter* MLST schemes, one devised by Bartual et al. (1), now referred to as the Oxford scheme, and the other is the Institut Pasteur scheme (2). Sequence type (ST) numbering is inevitably independent, and to avoid confusion, the scheme used should be stated; here we use ST_{Ox} and ST_{IP} to distinguish them. The databases for both of these schemes can be found at http://pubmlst.org/abaumannii. Each scheme uses seven genes, three of which are shared (Fig. 1). However, much of the chromosome is not sampled (Fig. 1), particularly in the Oxford scheme, where the seven genes are all in one half of the chromosome. In addition, some genes in each scheme (e.g., *rpoD*, *gltA*, and *cpn60* in the Oxford scheme or *gltA* and *cpn60* in the Institut Pasteur scheme) are close together (Fig. 1) and can potentially be replaced via a single recombination event.

Overall, the Institut Pasteur scheme more readily identifies members of clonal complexes (CCs), particularly CC1 and CC2, which correspond to global clone 1 (GC1) and GC2 (international clone [IC] I and IC II), the most important clones made up mainly of multiple-antibiotic-resistant isolates. In contrast, the Oxford scheme reveals the diversity in members of the same clone in the region of the capsule locus. The *gpi* gene is near one end of the capsule gene cluster, and variants that differ only in the *gpi* allele or double-locus variants that have different alleles for both *gpi* and the nearby *gyrB* gene are common. This variation, first noted by Hamouda et al. (3), was later traced to the existence of many recombinational replacements of this region that cause the structure of the capsular polysaccharide to differ (4).

A serious problem was revealed when STs in the Oxford scheme generated from whole-genome sequence data were compared to values determined with the primers specified by Bartual et al. (1). We first encountered a discrepancy between STs determined both ways in 2011 while analyzing GC2 isolate WM99c (5). We had determined the MLST profile of a closely related isolate, A91, as ST92 (1-3-3-2-2-7-3) (6). However, the genome sequence data indicated that WM99c was ST208 (1-3-3-2-2-97-3), which differs from ST92 by a single base at one end of the *gpi* gene. We sequenced the *gpi* amplicon generated by using the specified primers, and the sequence was for allele 7, and we published the ST of WM99c as ST92, rather than ST208 (5). Subsequently, we encountered similar problems with other isolates (Table 1). This led us to reexamine this issue, and we found that part of the forward primer sequence has been included in the region used for allele determination for the *gpi* gene (Fig. 2). The STs of all of the strains for which we had previously published ST_{Ox}s (Table 1) were reexamined by using

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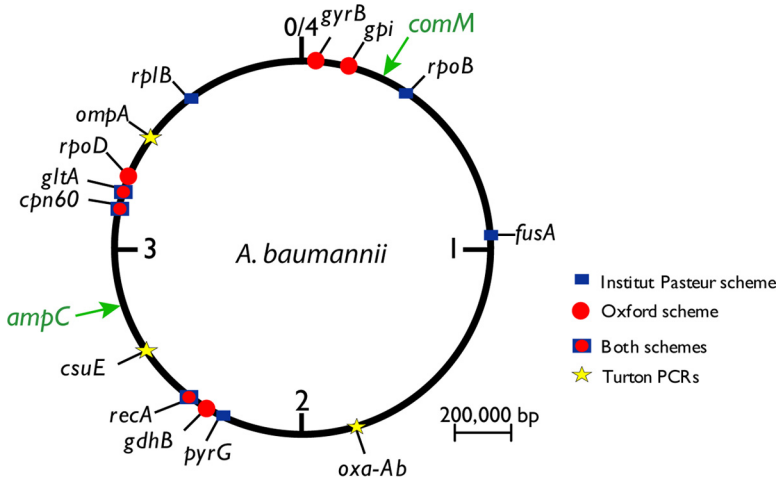

FIG 1 Circular map of the *A. baumannii* chromosome showing the locations of genes used in the Oxford and Institut Pasteur MLST schemes. Genes detected by trilocus typing by PCR (7) are also indicated by stars (Turton PCRs). The locations of the *comM* and *ampC* genes are also indicated.

whole-genome sequence data. In addition to ST92, which has been frequently reported for GC2 ($CC2_{IP}$) isolates and is, in fact, ST208, several were affected (Table 1). ST109, a commonly reported ST for GC1 ($CC1_{IP}$) isolates, was ST231. In all cases, the forward primer had a single base difference from the actual sequence (Fig. 2).

A broader investigation revealed that the amplification primers overlapped the region analyzed for another gene, namely, the reverse primer for the *cpn60* gene. We reported these problems to the curator of the MLST database, and subsequently, the primers amplifying these two genes were changed so that they lie outside the region used for allele determination. Unfortunately, these changes left the original problem in place. Because the regions analyzed were not altered to remove the problem primer sequences, many of the alleles and many STs in the Oxford database are not real. Prior reports or current ones where labs unaware of the change have continued using the original primers yield one ST while the genome sequence and the replacement primers yield another.

In our experience, the problem arises most often in the *gpi* gene, where the magnitude of the problem is amplified by the fact that the *gpi* allele lies within the capsule biosynthesis gene cluster. When the capsule locus is replaced, which is known to be a common occurrence (4), a different *gpi* sequence is introduced.

Hence, though the use of CC92 and CC109 continues, ST92 and ST109 may not actually exist. It would be useful if this problem were recorded on the MLST website.

TABLE 1 Discrepancies in Oxford MLST (*gltA-gyrB-gdhB-recA-cpn60-gpi-rpoD*)

Strain(s)	GC[a]	ST by original PCR method[b]	Reference(s)	ST determined *in silico* from genomes[b]	Reference
A1, G7, A297, WM98	1	109 (10-12-4-11-4-**9**-5)	8–11	231 (10-12-4-11-4-**98**-5)	12
D36	1	247 (10-12-4-11-4-**58**-5)	13–15	498 (10-12-4-11-4-**142**-5)	12
D78, D81	1	347 (10-12-4-11-4-**80**-5)	12	441 (10-12-4-11-4-**100**-5)	12
A85	1	126 (10-53-4-11-4-**64**-5)	13–15	781 (10-53-4-11-4-**200**-5)	12
A91, WM99c	2	92 (1-3-3-2-2-**7**-3)	5, 6, 16	208 (1-3-3-2-2-**97**-3)	17
RBH44	2	69 (1-46-3-2-2-**58**-3)	18	423 (1-46-3-2-2-**142**-3)	This study
A320	2	98 (1-12-3-2-2-**3**-3)	19	350 (1-12-3-2-2-**102**-3)	This study
D46		110 (1-15-2-28-1-**52**-32)	9	229 (1-15-2-28-1-**107**-32)	This study
RBH2 (F2)		125 (1-52-59-12-1-**18**-44)	9	1134 (1-52-59-12-1-**79**-44)	This study
RCH51		253 (1-52-29-28-18-**24**-7)	9	514 (1-52-29-28-18-**114**-7)	This study

[a]Global clones.
[b]Discrepancies are in bold type.

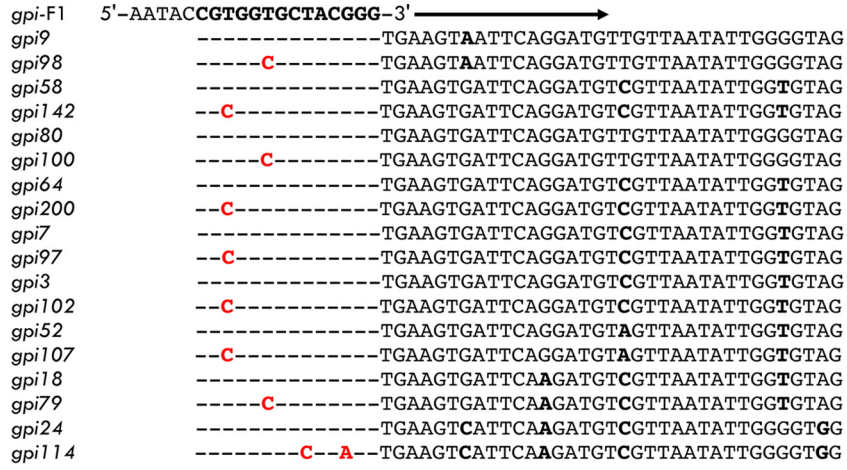

FIG 2 Comparison of actual sequences at the 5′ end of the *gpi* allele to that of the *gpi* forward primer, *gpi*-F1. The *gpi*-F1 primer sequence is shown above with the bases in the segment used as the *gpi* allele in bold. Sequences obtained with *gpi*-F1 are interspersed with the actual sequences with the correct base shown in red.

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